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RADIATION-STERILIZATION OF FOOD: A STATISTICAL ANALYSIS

by

Edward W. Ross, Jr.

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March 1973

UNITED STATES ARMY
NATICK LABORATORIES
Natick, Massachusetts 01760



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Radiation -- Sterilization of Food

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ABSTRACT

This report presents a mathematical analysis of the methods used for determining the effectiveness of radappertization (radiation-sterilization) of food. A general theory is developed which makes it clear that two inter-related distribution functions, the probability of organism death and the probability of can-sterilization, play important parts in the process. A critique is given of the Schmidt-Nank method for calculating the 12D dose and the implications of the experimental data are studied. Modifications in both experimental design and data analysis are proposed. These are evaluated by using them to analyze artificial data generated by a fairly realistic computer-simulation model. The proposed methods give considerably more accurate results than the traditional one, and it is concluded that the new methods appear promising for future use.

I. Introduction

This report is concerned with the determination of safe sterilization processes for canned food, i.e. processes which insure that the food is free of dangerous organisms. Although ionizing radiation is the method of sterilization considered here, the mathematical procedures described are equally applicable to any method of killing microorganisms in food.

We may summarize the present situation as follows. An expert committee of the United Nations Food and Agriculture, World Health Organization and the International Atomic Energy Agency has recommended a criterion of safety for radiation-sterilization (see [13]), which states that the probability must be no more than 1×10^{-12} that a dangerous microorganism (usually *Clostridium botulinum*) will survive the processing. The processing consists of exposing sealed cans of food to a dose of radiation under specified conditions, and the dose needed to satisfy the above criterion is called the 12D dose or minimal radiation dose (MRD). The 12D dose depends on both the microorganism and conditions (temperature, salinity pH, etc.) in the food substrate and is a measure of the radiation resistance of the microorganisms.

The presently accepted procedure for estimating the 12D dose follows the January 1971 recommendation of the National Academy of Science — National Research Council's Advisory Committee to Natick Laboratories on Microbiology of Food. The procedure consists of a set of experiments, collectively called an inoculated pack, and a computation based on the resulting data. The experiments consist of inoculating cans of food with spores of *C. botulinum*, sealing the cans and exposing them to doses of radiation. Typically, 10^7 spores are inoculated in each can, 100 replicate cans are exposed to each dose and the doses may range from 0 to 5 megarads in increments of .5 megarads. After irradiation all cans are incubated for six months at 30°C. The cans are examined for swelling weekly during the first month and monthly thereafter. At the end of incubation cans are tested for toxin presence, and all cans showing neither swelling nor toxin are subcultured for surviving spores. The computation takes the resulting partial spoilage data (usually based on surviving spores) and calculates the 12D dose by using the Schmidt-Nank formula [2].

The purpose of this report are, first, to describe the inadequacies of this procedure, and, second, to show that certain changes in both the experimental design and the calculation will permit a much improved estimate of the 12D dose. We emphasize two points about the modified procedure. First, worthwhile improvement is obtainable only when **both** the experimental design and the mathematical treatment are changed. Little gain in accuracy results from changing either alone. Second, since we are dealing with random processes, the 12D estimates depend on a decision about the form of the governing distribution function. The modified method greatly reduces the chance of an incorrect decision but does not completely remove it. It is still possible that a wrong decision, and hence an inaccurate estimate of the MRD, can result in any particular inoculated pack.

Some mathematical background is necessary for understanding this report. Most of the mathematics has to do with random variables, their associated distribution functions and the relations among them. This theory is put forth in Sections II and IV. Section III contains the critique of the Schmidt-Nank computation, and the remaining Sections describe the modified experimental and computational method and give examples of its use

II. General Theory

In this Section we give a simple probabilistic theory of spore sterilization and examine the conventional experiments in the light of this theory. The theory brings one main difficulty into clear view and suggests a way of dealing with it.

We assume that each spore in a given medium, irradiated under given conditions of temperature, pH etc. possesses a unique minimum lethal dose, X . If subjected to a dose above X , the spore will be inactivated, i.e. it will be unable to produce toxin and descendants; otherwise it will remain dangerous. The lethal dose, X , is a random variable, and we assume that it possesses a probability distribution function, $F(x)$,

$$F(x) = \text{Probability that } X \leq x, \quad (1)$$

and probability density function,

$$f(x) = dF(x)/dx. \quad (2)$$

Equation (1) means that $F(x)$ is the theoretical fraction of spores inactivated at dose x , and describes the behavior of individual spores under the conditions of the test. The 12D dose, which we call x_c , satisfies the equation

$$\begin{aligned} F(x_c) &= \text{Probability that } X \leq x_c = 1 - [\text{Probability that } X > x_c] \\ &= 1 - 10^{-12} \end{aligned} \quad (3)$$

In the experiments, n spores (typically $n = 10^6$) are put into a can and irradiated at the dose x under the test conditions. We say that a can is sterilized if all the spores in it are inactivated, and define Z_n as the minimum dose at which a can containing n spores is sterilized. Different cans will have different Z_n -values, hence Z_n is a random variable, just as X is. The distribution and density functions associated with Z_n are $\Phi_n(x)$, and $\phi_n(x)$,

$$\Phi_n(x) = \text{Probability that } Z_n \leq x \quad (4)$$

$$\phi_n(x) = d\Phi_n(x)/dx \quad (5)$$

Equation (4) means that $\Phi_n(x)$ is the theoretical fraction of cans sterilized at dose x .

There is a very important relationship between $\Phi_n(x)$ and $F(x)$, which we may derive as follows. Let X_1, X_2, \dots, X_n be the lethal doses of the n spores in the can. Since Z_n is the sterilizing dose, it is the largest number among X_1, X_2, \dots, X_n . Hence

$$Z_n \leq x$$

is logically equivalent to

$$(X_1 \leq x) \text{ and } (X_2 \leq x) \text{ and } \dots \text{ and } (X_n \leq x)$$

From (3) we have

$$\begin{aligned} \Phi_n(x) &= \text{Probability that } Z_n \leq x \\ &= \text{Probability that } (X_1 \leq x) \text{ and } (X_2 \leq x) \text{ and } \dots \text{ and } (X_n \leq x) \end{aligned}$$

We assume that the resistance of each spore is the same as if it were the only spore in the can, i.e. the spores act independently. Then the multiplicative law for the probability of independent events gives

$$\begin{aligned} \Phi_n(x) &= (\text{Probability that } X_1 \leq x) \cdot (\text{Probability that } X_2 \leq x) \cdot \\ &\quad \dots \cdot (\text{Probability that } X_n \leq x) \end{aligned}$$

Each spore obeys the same probability distribution, (1), hence

$$\begin{aligned} (\text{Probability that } X_1 \leq x) &= (\text{Probability that } X_2 \leq x) \\ &= \dots = (\text{Probability that } X_n \leq x) = F(x) \end{aligned}$$

Thus finally

$$\Phi_n(x) = \underbrace{F(x) \cdot F(x) \cdot \dots \cdot F(x)}_{n \text{ factors}} = [F(x)]^n \quad (6)$$

A somewhat different form of this relation is obtained by re-writing it as

$$\begin{aligned}\Phi_n(x) &= \{1 - [1 - F(x)]\}^n = \left\{1 - \frac{n[1 - F(x)]}{n}\right\}^n \\ &\approx e^{-n[1 - F(x)]},\end{aligned}\quad (7)$$

a result which is very accurate when $n \gg 1$ and $1 - F$ is small, which is almost always the situation when we need to know $\Phi_n(x)$. Solving (7) for $F(x)$ we obtain with great accuracy

$$F(x) \approx 1 + \frac{1}{n} \ln \Phi_n(x) \quad (8)$$

In addition we obtain from (5) and (6)

$$\phi_n(x) = n [F(x)]^{n-1} f(x). \quad (9)$$

Finally, it can be shown, see e.g. Gumbel [1], that

$$\Phi_n(x) \approx e^{-(e^{-\gamma})}, \quad \phi_n(x) \approx \alpha_n e^{-(\gamma + e^{-\gamma})} \quad (10)$$

$$\gamma = \alpha_n (x - U_n) \quad (11)$$

where U_n , the Characteristic Largest Value, and α_n , the Extremal Intensity Function, are found from

$$F(U_n) = 1 - n^{-1}, \quad \alpha_n = n f(U_n) = n \frac{dF(U_n)}{dx} \quad (12)$$

The distribution defined by (10) to (12) is called the extreme-value distribution derived from the distribution $F(x)$. Formulas (6) and (9) are exact, and (7) and (8) are such good approximations that they too may be regarded as exact for all practical purposes. Equations (10) are approximations to the exact relations (6) and (9) and are accurate when $|x - U_n|$ is not too large. The region where (10) is most accurate is the partial spoilage range, i.e. the x -values for which $\Phi_n(x)$ is near neither zero nor one. The quantities U_n and α_n^{-1} are approximate measures, respectively, of the location and width of the

partial spoilage range for cans containing n spores. As n increases, U_n (but not necessarily α_n^{-1}) increases, i.e. the partial spoilage range moves outward.

In the conventional inoculated pack N cans, each containing n spores, are exposed to a dose x , and after suitable incubation, counts are made of the number, $C(x)$, of cans that are sterilized or clean. Such a pack can be regarded as a sample of N cans, each of which has probability of sterilization $\Phi_n(x)$. It is well-known that the probability that exactly ξ cans will be sterilized is given by the binomial distribution,

$$\text{Probability that } [C(x) = \xi] = \frac{N!}{\xi!(N-\xi)!} [\Phi_n(x)]^\xi [1-\Phi_n(x)]^{N-\xi} \quad (13)$$

Moreover, the best estimate of $\Phi_n(x)$ that can be obtained from the data is

$$\hat{\Phi}_n(x) = \text{estimate of } \Phi_n(x) = \xi/N, \quad (14)$$

and, if $N \gg 1$, $\hat{\Phi}_n(x)$ is approximately normally distributed about its mean, $\Phi_n(x)$, with estimated standard deviation

$$\hat{\sigma}_{\hat{\Phi}} = [\hat{\Phi}_n (1-\hat{\Phi}_n)/N]^{1/2} \quad (15)$$

To summarize, we obtain from the conventional inoculated pack an experimental fraction, (14), of cans sterilized at dose x , and this is the best obtainable estimate of $\Phi_n(x)$. If packs are run at several different doses, we obtain several points on an experimentally-determined graph of $\Phi_n(x)$. There will be some scatter or noise in this graph, much of which is caused by the sampling error (i.e. the fact that $\hat{\Phi}_n(x) \neq \Phi_n(x)$) although some may also be due to random fluctuation in spore load, n , and dose, x . Formula (15) is an estimate of the scatter at dose x due to the sampling error.

Clearly, the inoculated pack provides quite a lot of information about $\Phi_n(x)$, especially if packs are run at several different doses. However, this information is of little use unless it leads to comparable information about $F(x)$, for it is $F(x)$ that enters the calculation of the 12D dose in Equation (3). In order to apply Equation (3), we have to know both the general form and parameter values of F . We shall see later that it is relatively easy to estimate the parameter values of F from data on $\Phi_n(x)$ if the general form of F is known, but it is not easy to find the general form of F .

This seems strange at first glance, for we can find $F(x)$ from $\Phi_n(x)$ directly by means of Equation (8). The difficulty arises because the doses at which $\Phi_n(x)$ is known are far out on the right-hand tail of the distribution $F(x)$. All probability distributions look very much alike in this region, and the scatter in $F(x)$ that arises from the scatter in the estimates of $\Phi_n(x)$ will make it very difficult to see the small differences between distributions.

This situation is sketched in Figure 1. Consider two distributions, $F_A(x; a_1, a_2)$ with general form A and parameters a_1 and a_2 , and $F_B(x; b_1, b_2)$, with general form B and parameters b_1 and b_2 . Suppose F_A is given. Then, because the general shape of F_B is quite similar to F_A , for $x \geq 3$ parameter values b_1 and b_2 can be found that will cause F_B and F_A to be nearly coincident in, say, the range $3.6 \leq x \leq 4.3$. If inoculated packs are run for $n = 10^6$, say, and the partial spoilage range is $3.6 \leq x \leq 4.3$, nearly the same partial spoilage results will be obtained from both distributions. Now, in real experiments we would know only $\Phi_n(x)$ in the partial spoilage range, and we could not tell whether the distribution function is F_A with parameters a_1 and a_2 or F_B with parameters b_1 and b_2 because both give about the same $\Phi_n(x)$ in the partial spoilage range.

Of course this difficulty does not arise if the form of $F(x)$ is known. It is usually assumed [3] that $F(x)$ is of simple exponential form. There is some (perhaps inconclusive) evidence to support this assumption when the spores are in a model system (i.e. a transparent, fluid substrate), see e.g. Anellis [4]. In Section III we shall show evidence against the assumption when spores are in a food.

Thus there is a need to determine $F(x)$ from measurements of $\Phi_n(x)$. Since the conventional inoculated pack was not designed for finding the form of $F(x)$, we should expect that other experimental designs may be superior for that purpose. Figure 1 shows that differences in distributions will be most visible when we have data over a wide range in x . The simplest way of obtaining this wide range is to test at several different spore loads, i.e. values of n , because the partial spoilage range moves outward as n increases. This is shown in Figure 2, where we see that, although F_A and F_B may coincide over the partial spoilage range for $n = 10^6$, they will not also coincide over the partial spoilage range for $n = 10^3$. Thus, if tests are made at both spore loads, the resulting partial spoilage data has a much better chance of showing differences between F_A and F_B than would a test at either $n = 10^3$ or 10^6 alone.

We shall return to the problem of identifying the form of $F(x)$ and determining its parameters in Sections V and VI.

III. A Critique of the Schmidt-Nank Calculation

This Section contains a sketch and critique of the Schmidt-Nank procedure for estimating the 12D dose.

The experimental procedure for a conventional inoculated pack has been described in the previous Sections. The resulting data is $\hat{\Phi}_n(x)$, see Equation (14), evaluated at one or more x -values. The Schmidt-Nank Procedure for estimating the 12D dose is based primarily on the assumption that $F(x)$ is of simple exponential form,

$$F(x) = 1 - e^{-\lambda x} \quad (16)$$

If N is the number of cans tested at dose x , n is the number of spores in each can and R is the total number of surviving spores, then some simple manipulations show that x_c can be estimated from

$$\hat{x}_c = 12\hat{D} \quad (17)$$

$$\hat{D} = \frac{x}{\log_{10} (Nn) - \log_{10} R} \quad (18)$$

Here \hat{D} is the estimated value of D , the decimating dose, i.e. dose at which the probability of spore death is

$$F(D) = 9/10$$

These formulas are not very useful as they stand because there is no practical way to measure R . In the Schmidt-Nank Method this difficulty is overcome by a second assumption, namely that exactly one spore survives in every can that is spoiled (not sterilized), i.e.

$$R = N - \xi = N(1 - \frac{\xi}{N}), \quad (19)$$

or, using (14),

$$R = N[1 - \hat{\Phi}(x)], \quad (20)$$

if ξ out of N cans are sterilized at dose x . This estimate of R is used in Equation (18) and permits the evaluation of \hat{D} and hence \hat{x}_c .

The Schmidt-Nank Formula, (18), has been generally accepted as a simple, standard method for estimating the 12D dose. However, in recent years other procedures have been suggested as alternatives to the Schmidt-Nank Formula, e.g. [5] and [6]. This is evidence of growing uneasiness about the accuracy of the method, but no systematic study of its validity has appeared. We shall present one in the ensuing paragraphs.

The principal criticisms that can be levelled against the Schmidt-Nank Procedure are these four (some of which are related to each other).

- (i) The assumption of an exponential distribution may be wrong.
- (ii) The assumption that one spore survives in each can that is not sterilized is questionable.
- (iii) The results of using the method on experimental data are inconsistent with the assumptions.
- (iv) The procedure is confusing and unclear.

We shall examine these criticisms below, but let us begin by recalling that a good mathematical theory should be in agreement with observations, clear, as simple as possible and should heighten our understanding of the biological process it describes.

First, there is much experimental evidence that something is wrong with the Schmidt-Nank Formula. For, if it is applied to experimental data at several different doses, it gives an estimate of D (and hence x_c) derived from each test dose. If the theory is correct, the same D should be obtained from each test dose, aside from random fluctuations. A typical set of experimental results [5], is reproduced in Table 1. It is clear from this and other data [7], [8], [9] and [10], that the estimate of D increases very markedly as x increases. This trend is unambiguous and far too pervasive to be attributed to any sort of randomness. It is completely at odds with the theory although it is hard to discern whether assumptions (i) or (ii) or both are at fault. This trend is also observable in thermal sterilization processes, see [1].

TABLE I

Radiation resistance of representative strains of C. botulinum spores in cured Ham

Strain	Dose	No. of cans with viable <u>C. botulinum</u>	Schmidt-Nank D-value
33A	1.0	17/20	.148
	1.5	15/20	.220
	2.0	8/20	.282
	2.5	1/100	.288
77A	1.0	16/20	.167
	1.5	11/20	.245
	2.0	5/20	.309
12885A	1.0	19/20	.149
	1.5	5/20	.206
	2.0	3/20	.267
	3.0	1/100	.346
41B	.5	18/20	.072
	1.0	13/20	.142
	1.5	6/20	.203
	2.5	1/100	.282
53B	.5	19/20	.071
	1.0	14/20	.140
	1.5	8/20	.203
	2.0	1/20	.241

Second, the assumption (ii) implies a relation between $\Phi_n(x)$ and $F(x)$ that is different from (6). To see this, we notice that assumption (ii) implies that only two outcomes of a can-sterilization experiment are possible, namely either (a) the can is sterilized or (b) exactly one spore survives in it. Hence, on this assumption

$$1 - \Phi_n(x) = \text{theoretical fraction of cans in which exactly one spore survives.}$$

Since N cans are irradiated, the total theoretical number of surviving spores is $N[1 - \Phi_n(x)]$ out of Nn spores exposed. The fraction of spores surviving is

$$\frac{N[1 - \Phi_n(x)]}{Nn} = 1 - \text{fraction of spores killed} = 1 - F(x)$$

Therefore we would obtain

$$\Phi_n(x) = 1 - n[1 - F(x)] \quad \text{or} \quad F(x) = 1 - n^{-1}[1 - \Phi_n(x)] \quad (21)$$

instead of (6), as a consequence of assumption (ii). Equation (6) was derived from the reasonable assumption that the minimum sterilizing dose for a can is the minimum lethal dose for the most resistant spore in the can. Assumption (ii) therefore gives results that disagree with that assumption in general, and must be logically doubtful.

Moreover, it is clear that the true total number of spores surviving radiation, R , is greater than (or equal to) the number $N - \xi$, given by Assumption (ii), Equation (19). Equation (18) shows that an increase in R causes an increase in D , hence the true D -value is larger than that given by (18). However, the difference between these two D -values is usually not very great because almost always $R \ll Nn$.

Finally the Schmidt-Nank calculation is confusing because in deriving it the authors did not give any clear indication that two distinct distributions, $F(x)$ and $\Phi_n(x)$, are involved. The formulas show it, Equations (18) and (20), but the failure to point it out explicitly has led others into confusion when trying to modify the calculation.

For example [5] describes an attempt (by Weibull plotting) to ascertain the form of the partial spoilage distribution, i.e. $\Phi_n(x)$. The conclusion, that the distribution was nearly normal, is not inconsistent with the form (10). However, the interpretation was marred by a number of confusing statements, evidently arising from failure to distinguish $F(x)$ from $\Phi_n(x)$.

Still another example concerns the relation between the LD50 and the D dose. The LD50 is commonly called the median of a distribution, and we shall designate it by x_h . Any distribution, say $G(x)$, possesses a median and a D-value defined by

$$G(x_h) = 1/2$$

$$G(D) = 9/10$$

Thus the median and D dose for the can distribution, $\Phi_n(x)$, satisfy

$$\Phi_n(x_h) = 1/2 \quad \text{and} \quad \Phi_n(d) = 9/10$$

and the median and D dose for the spore distribution satisfy

$$F(x_h) = 1/2 \quad \text{and} \quad F_n(D) = 9/10.$$

For each distribution there is a well-defined relation between the D-dose and the LD50 dose, x_h , and this relation is different for different distributions. However, the relation that is commonly used in food-sterilization is between the LD50 of the can distribution, $\Phi_h(x)$, and the D of the spore distribution, $F(x)$. Although most writers have derived the correct relationships between D and LD50, none has emphasized that they refer to two different distributions, probably because they did not clearly perceive that two distributions were involved.

The general relation between D and x_h (or LD50) can be found only if $F(x)$ is known. By definition we have

$$F(D) = 9/10 \quad \text{and} \quad \Phi_n(x_h) = 1/2,$$

and from (8) we obtain

$$F(x_h) = 1 + \frac{1}{n} \ln (1/2).$$

We can solve these relations if F is known,

$$D = F^{-1} (9/10)$$

$$x_h = F^{-1} [1 + \frac{1}{n} \ln (1/2)]$$

and obtain

$$\frac{D}{x_h} = \frac{F^{-1}(9/10)}{F^{-1}\left[1 + \frac{1}{n} \ln(1/2)\right]}$$

For example, if F is of exponential type, Equation (16), then

$$D = \frac{1}{\lambda} \ln_e 10$$

$$x_h = \frac{1}{\lambda} [\ln n - \ln(\ln 2)]$$

$$D/x_h = \frac{1}{\log_{10} n - \log_{10} .693},$$

a result which agrees with that given earlier by Schmidt-Nank (12).

To summarize, the most telling criticism of the Schmidt-Nank computation is that its results contradict the assumption that D is a constant. Another valid general criticism is that the failure to see that two distributions, rather than one, are involved, can cause considerable confusion, as illustrated in the preceding examples. This has undoubtedly handicapped attempts to improve on the Schmidt-Nank Procedure. The specific assumption (ii), p. 10, is illogical and should be abandoned, but it does not usually cause large errors in the estimate of D . The assumption, (i), of an exponential distribution is cast into doubt by the experimental evidence that D is not constant.

It appears therefore that both the experimental design of the inoculated pack and the procedure for estimating the D -value should be modified.

IV. Implications of Experimental Results

In the preceding Section we have pointed out that many inoculated pack results show that D , estimated by Equation (18), increases with an increase in dose, x . There, this trend was cited as evidence that the method of estimating D was faulty. In this Section we shall see what this trend tells us about the form of $F(x)$ when the general theory of Section II is used instead of the presently accepted theory. We shall derive a general condition that $F(x)$ must satisfy if it is to cause the observed increase in D . Then we shall apply this to the most common forms of distributions, to see which of them satisfies the condition. Quite a lot of detailed mathematics is needed in this Section, most of which is merely sketched here but is given in Appendix A.

We begin with Formula (18). The usual Schmidt-Nank Estimate is obtained by combining (18) and (20) and simplifying so that

$$\hat{D} = x \{ \log_{10} n - \log_{10} [1 - \hat{\Phi}(x)] \}^{-1}$$

The theoretical value of D is therefore merely

$$D = x \{ \log_{10} n - \log_{10} [1 - \Phi(x)] \}^{-1} \quad (22)$$

The experimental results, derived from tests in the partial spoilage range, show that D almost always increases with x . Therefore we shall study the behavior of the theoretical D , given by (22), and see under what conditions it increases throughout the partial spoilage range. The condition that this D increase is.

$$dD/dx = 2.303 A(x)/B^2(x) > 0,$$

where

$$A(x) = \log_e n - \log_e [1 - \Phi(x)] - x [1 - \Phi(x)]^{-1} (d\Phi/dx)$$

$$B(x) = \log_e n - \log_e [1 - \Phi(x)],$$

and this condition must hold throughout the partial spoilage range. Since $B^2(x)$ is always positive, the condition that D increase is equivalent to

$$A(x) > 0 \quad (23)$$

in the partial spoilage range. The approximate formulas (10) and (11) are accurate in the partial spoilage range, and we may use them in analyzing the behavior of Equation (23).

The details of this analysis are given in Appendix A. The conclusion is that $D(x)$ increases monotonically with x in the partial spoilage range if and only if

$$\alpha_n U_n \leq \log_e n. \quad (24)$$

The general appearance of $D(x)$ in the partial spoilage range when $\alpha_n U_n \leq \log_e n$ and $\alpha_n U_n > \log_e n$ is shown in Figure 3. As x increases, dD/dx is always positive at the lower end of the partial spoilage range, but, if $\alpha_n U_n \leq \log_e n$, it reaches a maximum and thereafter decreases.

This tells us several things. First, D always increases at the lower end of the partial spoilage range. This agrees with the experiments but does not give us any information about $F(x)$. If D also increases in the upper portion of the partial spoilage range, then we can conclude that the inequality (24) must be satisfied. Examining the data of Table 1, we see a lot of cases where D increases in the upper part of the partial spoilage range, and data from other sources (7), (8), (9) and (10) shows the same behavior. We conclude, therefore, that under most circumstances the distribution $F(x)$ must be such that the inequality (24) is satisfied.

We shall now consider several of the common distributions and see whether there are parameter values for which (24) is satisfied. The distributions to be studied are listed below.

(i) Weibull Distribution

The distribution function is

$$\begin{aligned} F(x) &= 0 & x < 0 \\ &= 1 - \exp \left\{ - (x/\eta)^\beta \right\} & x > 0 \end{aligned}$$

where $\eta > 0$ and $\beta > 0$. From Equations (12) we find

$$\begin{aligned} U_n &= \eta (\log_e n)^{1/\beta} \\ \alpha_n &= (\beta/\eta) (\log_e n)^{(1-\beta^{-1})} \end{aligned}$$

and hence

$$\alpha_n U_n - \log_e n = (\beta - 1) \log_e n$$

In order that (24) be satisfied, we must have

$$\beta \leq 1.$$

Now when $\beta \approx 1$ this distribution reduces to the Exponential Distribution, and when $\beta \approx 3.3$ this distribution resembles a Normal Distribution in the sense that certain moments are the same as for a Normal Distribution. We see that an Exponential Distribution barely satisfies the inequality (24), indicating that D is nearly at the upper end of the partial spoilage range for the Exponential Distribution. In practical terms this means that if the distribution is Exponential, the experimentally obtained values of $D(x)$, $\hat{D}(x)$, may fluctuate instead of increasing in the upper part of the partial spoilage range because randomness due to sampling and other errors may outweigh the small increases in the theoretical D . The situation is illustrated in Figure 4, where we see in the upper sketch that $\hat{D}(x_1) > \hat{D}(x_2)$ because of the randomness in \hat{D} for an Exponential Distribution. This is less likely to happen in cases where $U_n \alpha_n < +\log_e n$, as illustrated in the lower sketch of Figure 4, although it could happen if $\log_e n$ is only slightly larger than $\alpha_n U_n$ for the distribution.

(ii) Normal Distribution

The Normal Distribution Function is

$$F(x) = \frac{1}{\sigma\sqrt{2\pi}} \int_{-\infty}^x e^{-\frac{1}{2}\left(\frac{t-a}{\sigma}\right)^2} dt$$

and is rather awkward to analyze because as n becomes large α_n and U_n attain their limiting behavior very slowly. However, α_n and U_n are tabulated in Gumbel [2], and a few simple calculations show that

$$\alpha_n U_n > \ln n \quad \text{when} \quad n > 20.$$

This result is not too helpful because spores cannot have a truly normal distribution. The reason is that we must have $F(x)=0$ when $x<0$, i.e. negative doses are impossible. Therefore, only normal distributions truncated at $x=0$ are realistic. The truncation introduces extra complication into the evaluation of U_n and α_n , and we shall omit the details for the sake of brevity. The final result is the same as before, i.e.

$$\alpha_n U_n > \log_e n$$

for a normal distribution truncated at $x=0$.

(iii) The Lognormal Distribution

The lognormal distribution has

$$\begin{aligned} F(x) &= 0 & x < 0 \\ &= F_g[\beta \log_e(x/\eta)] & x > 0, \end{aligned}$$

where F_g is the standardized normal distribution function

$$F_g(z) = (2\pi)^{-1/2} \int_{-\infty}^z e^{-1/2 x^2} dx.$$

If U_g and α_g are the U_n and α_n for the distribution function F_g , then Gumbel shows that

$$\begin{aligned} U_n &= \eta e^{U_g/\beta} \\ \alpha_n &= \frac{\beta \alpha_g}{\eta} e^{-U_g/\beta} \end{aligned}$$

and therefore

$$\alpha_n U_n = \beta \alpha_g.$$

The lognormal distribution satisfies the condition (24) when

$$\beta \alpha_g < \log_e n$$

Figure 5 is a graph of the largest acceptable value of β as a function of n .

Other distributions, such as the Gamma or Chi-Square distributions, could also be studied, but their theory is more difficult than the three we have discussed, and there is no compelling reason to think that spores obey those distributions. We shall therefore limit ourselves to the three distribution already discussed.

Of these, the normal distribution can be discarded because it does not lead to Schmidt-Nank D-values that increase with x in the partial spoilage range. We come to the conclusion that the likeliest candidates for the distribution of spore inactivation doses are the Weibull and lognormal distributions, and we shall therefore concentrate on methods for deciding which of these is closer to the true distribution.

V. A New Method for Finding the Distribution Functions and 12D Dose

In this Section we shall present a general method for determining the form and parameters of the distribution function $F(x)$ from measurements $\hat{\Phi}_n(x)$. The basic idea is a very simple and familiar one. We hypothesize that we have a certain form of distribution, F , and we subject the data $\hat{\Phi}_n(x)$ to a transformation which would reduce the data plot to a straight line if F were of the assumed form. The straightness (absence of curvature) of the plot is a measure of how well the data supports the hypothesis about the form of F , and the slope and intercept of the line provide estimates of the parameters of the distribution. In practice we usually consider several competing forms of $F(x)$, so that we subject the data to several different transformations, one appropriate for each of the competing forms. The form whose transformation produces the straightest plot is the one which fits the data best.

We illustrate the procedure by deriving the formulas appropriate to the two strongest candidates, the Weibull and lognormal distributions.

a. Basic Formulas

When $x > 0$, the form of the Weibull distribution is

$$F(x) = 1 - \exp\left\{- (x/\eta_w)^{\beta_w}\right\},$$

where η_w and β_w are the parameters. We combine this with Equation (8) to obtain

$$\exp\left\{- (x/\eta_w)^{\beta_w}\right\} = -n^{-1} \log_e \Phi_n$$

or

$$(x/\eta_w)^{\beta_w} = \lambda + \log_e n.$$

$$\lambda = -\log_e (-\log_e \Phi_n)$$

We take logarithms again and get

$$\beta_w [\log_e x - \log_e \eta_w] = \log_e [\lambda + \log_e n].$$

From this we see that, if we define the transformation

$$\begin{aligned} y_W &= \log_e \{ \log_e n - \log_e (-\log_e \Phi_n) \} \\ t &= \log_e x, \end{aligned}$$

then we obtain the straight line relation between y_W and t ,

$$y_W = \beta_W t - \beta_W \log_e \eta_W, \quad (25)$$

provided that F is a Weibull distribution with parameters β_W and η_W . This is the desired transformation.

For the lognormal distribution, the form when $x > 0$ is

$$F(x) = F_g [\beta_L \log_e (x/\eta_L)]$$

where β_L and η_L are the parameters. Omitting the details we find that the transformation

$$\begin{aligned} y_L &= F_g^{-1} [1 + \frac{1}{n} \log_e \Phi_n] \\ t &= \log_e x \end{aligned}$$

leads to the straight line relation

$$y_L = \beta_L t - \beta_L \log_e \eta_L, \quad (26)$$

if F is a lognormal distribution with parameters β_L and η_L .

In practice we do not know $\Phi_n(x)$. In its place we use the quantities $\hat{\Phi}_n(x_m)$, the experimentally obtained fractions of cans sterilized (see Equation (14)), at the test doses x_m , $m = 1, 2, \dots, M$. To decide whether the true F has Weibull or lognormal form, we construct two graphs of the data points versus $\log_e x$, using Formulas (25)

and (26), respectively. The transformation which produces the straighter graph of the data points corresponds to the likeliest form of F. Then, if the plot is of the form

$$y = A + B t,$$

we obtain the estimated parameters

$$\hat{\beta} = B \quad (27)$$

$$\hat{\eta} = e^{-(A/B)} \quad (28)$$

for whichever distribution has been chosen. The 12D dose, x_c , is estimated using

$$\hat{x}_{cw} = \hat{\eta}_w (27.63)^{(1/\hat{\beta}_w)} \quad (29)$$

if the Weibull Distribution has been selected and

$$\hat{x}_{cL} = \hat{\eta}_L e^{(7.0345/\hat{\beta}_L)} \quad (30)$$

if the lognormal has been chosen.

b. Experimental Design

In theory the above is easy enough but in practice it is often difficult to tell by eye which of the two plots is straighter, especially since there is noise (random fluctuations) in the data. The discussion in the latter portion of Section II leads us to expect that both plots will appear nearly straight if they cover only the range of x corresponding to the partial spoilage range for, say, $n = 10^7$. Figure 6 shows this very clearly. It contains the two graphs for the case where $\Phi_n(x)$ has exactly the theoretical values, $\Phi_n(x)$, derived from a lognormal distribution with $\beta_L = 2$ and $\eta_L = .2$ when $n = 10^7$. The graph given by the lognormal transformation (the upper set of points in Figure 6) is exactly straight. The graph given by the Weibull transformation (the lower set of points) is not exactly straight, but the curvature is so slight that it is hard to see which graph is straighter. If we were given only the data, we could not tell whether the distribution is lognormal with $\beta_L = 2$ and $\eta_L = .2$ or Weibull with $\beta_w = .664$ and $\eta_w = .0409$. Moreover, we see from Equation (27) and (28) that the 12D dose is estimated to be 6.74 if the distribution is lognormal (as it really is) and 6.06 if it is (incorrectly) thought

to be of Weibull type. The large difference between these estimates of 12D attests to the importance of finding the distribution form correctly.

The discussion at the end of Section II shows that we can get around this difficulty by conducting tests at several different spore loads. This is illustrated in Figure 7, where, as in Figure 6, the data is assumed to have exactly the theoretical values derived from a lognormal distribution with $\beta_L = 2$ and $\eta_L = .2$. However, we assume now that the partial spoilage data has been taken at three different spore loads, $n = 10^3, 10^5, 10^7$. The upper graph for the lognormal distribution is exactly straight. The curvature in the lower graph, although not overwhelming, is rather easily visible, certainly much more so than in Figure 6, which is now reproduced as the portion of Figure 7 for $n = 10^7$.

If we examine Figure 7 carefully, we see that the curvature in the Weibull graph is more noticeable at smaller values of t than at larger ones. That is, the difference between the Weibull and lognormal distributions is easier to see, the lower is the spore load. This is in agreement with the idea that different distributions tend to look more alike as x becomes larger, hence the differences should be more visible for small x , i.e. at low spore loads. This suggests that we should use extremely small spore loads (10^1 or 10^2 spores per can) if we want to see the differences between distributions very clearly. However, there are two reasons for not doing so. First it is impractical to measure the numbers of spores when very few are present. Second, the extrapolation to find the 12D dose is very coarse if we have results only for small spore loads. The best compromise is to conduct tests at several different spore loads.

It is not easy to give a simple general rule for deciding how many different spore loads, and which spore loads, should be used. The best choice depends on the form and parameters of $F(x)$, which are not known before the tests are run. However, usually we want the widest possible range in x -values, and hence in spore loads, which means that two of the spore loads should be the highest and lowest practicable values, i.e. roughly $n = 10^3$ and $n = 10^7$ because of current experimental limitations. For many practical distributions these two spore loads will have partial spoilage ranges that are far apart, and it is desirable that the partial spoilage ranges should almost overlap as in Figure 7, so it is natural to use an intermediate spore load, $n = 10^5$. Using still more intermediate spore loads like $n = 10^4$ and 10^6 will often give partial spoilage ranges that overlap each other, which is inefficient. As a general rule, therefore, we suggest using three spore loads, $n = 10^3, 10^5$ and 10^7 , as was done in the example of Figure 7.

c. The Computation Scheme

In order to choose the likeliest form of F from test data, we have to decide which of the two graphs is the straighter. We have seen in Figure 7 that, even when tests are run at several spore loads, the curvature in the "incorrect" graph may not be great. Moreover, in practice the situation will be worse than shown there because of the random errors in the data. It is most desirable to have a sensitive analytical test for measuring the curvature of the graphs, rather than relying on the unaided eye.

The computational method consists of approximating the data, using the Least-Squares procedure, by means of a second degree polynomial,

$$Y_W = A_W + B_W t + K_W t^2 \quad (31)$$

$$Y_L = A_L + B_L t + K_L t^2, \quad (32)$$

the data having been first transformed by the appropriate Weibull and lognormal relations, Equations (25) and (26). The method (described in Appendix B), gives estimates of the constants A_W, B_W, K_W and A_L, B_L, K_L and also gives the squared sums of errors, S_W^2 and S_L^2 , of the approximations. The estimates of the parameters are found from Equations (27) and (2B),

$$\hat{\beta}_W = B_W, \quad \hat{\eta}_W = e^{-(A_W/B_W)} \quad (33)$$

$$\hat{\beta}_L = B_L, \quad \hat{\eta}_L = e^{-(A_L/B_L)}. \quad (34)$$

As measures of the curvatures of the plots, we use

$$\hat{\rho}_W = K_W/S_W, \quad \hat{\rho}_L = K_L/S_L. \quad (35)$$

If $|\hat{\rho}_W| < |\hat{\rho}_L|$, then we conclude that $F(x)$ is a Weibull distribution with parameters given by Equations (33) and 12D dose as in Equation (29). If $|\hat{\rho}_L| < |\hat{\rho}_W|$, we conclude that $F(x)$ is of lognormal form with parameters given by Equation (34) and 12D dose of Equation (30).

The experimental design described in part (b) and the computational scheme outlined above are the methods we suggest as replacements for the conventional inoculated pack

and Schmidt-Nank Formula. We shall demonstrate the superiority of the proposed method in the next Section, but here we wish to caution the reader that, even if the new method is used, there is a (usually small) probability that either no conclusion or a wrong conclusion about the form of $F(x)$ will be reached. This is inevitable because of the randomness in the data and smallness of the differences we are trying to find. We shall discuss this probability in the next Section.

VI. Computer Simulation of the Inoculated Pack

In this Section we describe a Monte-Carlo method for generating artificial but reasonably realistic experimental data, like that obtained from both the conventional inoculated pack and the revised experiments proposed in Section V. By using this data in the Schmidt-Nank Formula and the computation scheme of Section V, we can assess the performance of both procedures in a situation where we know what the "true" answers are. In particular we are concerned with the following, inter-related, questions.

- (i) Does the revised experimental design permit more reliable determination of the form of $F(x)$ than the conventional design?
- (ii) If so, how reliable is the new method?
- (iii) What are the relative accuracies of the 12D estimates obtained by the new method and the conventional procedure.

The Monte-Carlo simulation can provide answers to other questions as well, but we shall concentrate on the above three.

a. Description of the Simulation Model.

The simulation model is a Fortran IV computer program that generates artificial test data. The user gives the program the following information

- (i) The function $F(x)$
- (ii) The intended spore loads, n_1, n_2, \dots, n_L
- (iii) The intended doses at each spore load,
 $x_{11}, x_{12}, \dots, x_{1k}$ at spore load n_1
 $x_{21}, x_{22}, \dots, x_{2k}$ at spore load n_2
 $x_{L1}, x_{L2}, \dots, x_{Lk}$ at spore load n_L

- (iv) The number of cans tested at each dose, N .
- (v) The standard deviation in each spore load, $\sigma_{n_1}, \sigma_{n_2}, \dots, \sigma_{n_L}$
- (vi) The standard deviation in dose, σ_x .

For each can that is tested two random numbers, (normally distributed with zero mean) are generated, i.e. the error in spore load and the error in dose. These are added to the intended spore load and dose, respectively, to get the dose and spore load at which the test is actually run. Equation (6) then gives the theoretical probability that the can is sterilized. Another random number, uniformly distributed between zero and one, is generated, and the can is taken to be sterilized if this number is less than the theoretical probability.

Counts are made of the numbers of cans sterilized at each dose and spore load, and the final totals are printed out. These results are then entered into a program for computing the Schmidt-Nank estimate of the 12D dose for each partial spoilage dose, as well as a longer program that estimates $\hat{\beta}_W, \hat{\eta}_W, \hat{\rho}_W, \hat{x}_{cW}$ and $\hat{\beta}_L, \hat{\eta}_L, \hat{\rho}_L, \hat{x}_{cL}$ from all the partial spoilage data. Our computation procedure is such that the form of the distribution (i.e. Weibull or lognormal) should be that giving the smaller value of $|\rho|$. Having decided the form of $F(x)$, we then take β, η and the 12 D estimate, x_c , to be those for the chosen distribution.

The form of $F(x)$ selected by our computation procedure should always agree with what we assumed as input (i) if there were no random errors. In reality, of course, random errors are always present and will sometimes cause the computation procedure to choose the wrong form, leading to a bad estimate of the 12D dose. The probability of this mistaken identification of $F(x)$ is one of the main quantities that we want to study.

b. Description of the Tests.

Because NLABS was very concerned in this period with radiation resistance of beef at -30°C , the main numerical experiments were conducted on a Weibull and a lognormal distribution with the following sets of parameters:

$$\begin{aligned}\beta_W &= .856, & \eta_W &= .1115, & x_{cW} &= 5.38 \\ \beta_L &= 2.334 & \eta_L &= .2925, & x_{cL} &= 5.96\end{aligned}$$

These are two distributions that roughly imitate the results of a small inoculated pack that was carried out at NLABS. We shall take them as typical of their respective distribution types for the case where beef is tested at -30°C .

The basic standard errors in dose and spore load were then taken as

$$\sigma_x = .02x$$

$$\sigma_{n_1} = \sigma_{n_2} = \sigma_{n_3} = \sigma_n = .1 n$$

which, since the distribution of these errors is normal, means roughly that the greatest error in dose is less than $\pm 6\%$ of the dose and the greatest error in spore load is less than about $\pm 30\%$ of the spore load. These are typical of the error magnitudes that often occur in real inoculated packs.

In order to make the numerical experiments as uniform as possible, the main series of numerical experiments were run at the doses shown below,

$$\begin{array}{llllllll} n = 10^7, & x = & 2.6, & 2.8, & 3.0, & 3.2, & 3.4, & 3.6 \\ n = 10^5, & x = & 1.7, & 1.9, & 2.1, & 2.3, & 2.5, & 2.7 \quad (36) \\ n = 10^3, & x = & .7, & .9, & 1.1, & 1.3, & 1.5, & 1.7. \end{array}$$

In this series the number of cans, N , at each dose was varied through the values $N = 10, 20, 30$ and 40 , that is the total number of cans tested in each case varied through $180, 360, 540$ and 720 , respectively. We denote these cases as $W10, W20, W30$ and $W40$ when $F(x)$ was given the Weibull form and $L10, L20, L30$ and $L40$ when $F(x)$ was given the lognormal form.

Many repetitions of each case were run and counts were made of the instances in which the computational procedure correctly identified the distributions $F(x)$. Also in two cases records were kept of the estimated 12D when the distribution was both correctly and incorrectly identified, and statistics were compiled concerning the average and variance of the estimated 12D in these cases. From this series of tests it is possible to get an idea about how reliably the form of $F(x)$ is identified by the proposed method, and how accurate the 12D estimate is, for different numbers of cans tested.

Another set of cases was also run, in which $N = 120$ cans at each dose, a single spore load, $n = 10^7$, was used and the doses were

$$x = 2.6, \quad 2.8, \quad 3.0, \quad 3.2, \quad 3.4, \quad 3.6.$$

These cases were designated W120 or L120 when $F(x)$ was of Weibull or lognormal form respectively. If we compare W120 with W40 and L120 with L40 we see that both have the same total number of cans (720), and differ only in that W40 contains tests at three spore loads with 40 cans at each dose, while W120 contains tests at only one spore load with 120 cans at each dose (and similarly for L40 and L120). The comparison of these results gives an answer to the question, "Is it more efficient to test a given number of cans at different spore loads or to use only a single spore load but a larger number of cans at each dose?"

Finally the special case, designated E40, where the distribution is Weibull with $\beta = 1$ and $\eta = .165$ was run with $N = 40$ cans per dose at the spore loads and doses of Formula (35). When $\beta = 1$, the Weibull distribution becomes an exponential, and we are therefore testing a case where the Schmidt-Nank method should give its most accurate predictions of the 12D dose.

The results of these cases are described in the next subsection.

c. Results of the Artificial Tests

We first show an example of the data and estimations that result from a typical run of case W40. Next we describe the results of the main series of tests, then the comparisons of W40 and L40 with W120 and L120, and finally case E40.

A fairly typical set of artificial data for the case C_{W40} (i.e. a Weibull distribution, tested at 40 cans/dose) is shown in table 2, which also lists the important estimated quantities, including the 12D estimate given by the Schmidt-Nank Formula (18) at each dose. Figure 8 presents graphs of y_W and y_L , calculated according to Equations (25) and (26).

Since

$$|\rho_W| = .67 < |\rho_L| = 2.42$$

TABLE 2

Results of a typical run of case W40.

Spore load n	Dose	Fraction of cans sterilized	Schmidt-Nank 12D
10^7	2.6	0/40	—
	2.8	10/40	4.72
	3.0	26/40	4.83
	3.2	36/40	4.80
	3.4	38/40	4.92
	3.6	39/40	5.02
10^5	1.7	0/40	—
	1.9	7/40	4.49
	2.1	29/40	4.53
	2.3	33/40	4.79
	2.5	39/40	4.54
	2.7	38/40	5.14
10^3	.7	0/40	—
	.9	4/40	3.55
	1.1	15/40	4.12
	1.3	28/40	4.43
	1.5	36/40	4.50
	1.7	39/40	4.43

$$\hat{\rho}_W = .67, \quad \hat{\rho}_L = 2.42$$

$$\hat{\beta}_W = .876, \quad \hat{\eta}_W = .1187, \quad \hat{x}_{CW} = 5.25$$

$$\hat{\beta}_L = .4451, \quad \hat{\eta}_L = .2803, \quad \hat{x}_{CL} = 6.42$$

the computation method tells us that the data comes from a Weibull Distribution, which is correct. The predicted 12D dose is $\hat{x}_{CW} = 5.25$, and we know that the true 12D is 5.38, hence in this case the error in the estimated 12D is less than 3%, which is quite good. The Schmidt-Nank procedure of course gives no method for determining the distribution, (it assumes an exponential form), but it gives estimates of 12D ranging from 4.72 to 5.02 with average 4.86, if we use only the data for $n = 10^7$. This is much poorer than the estimate obtained by the proposed method, 5.25. There is considerable noise (i.e. scatter) in the plotted points of Figure 8. This makes it a little difficult to see that the lognormal graph is more curved than the Weibull plot, but the relative magnitudes of ρ_W and ρ_L give a clear signal that this is so.

The results of the main series of tests are shown in Table 3 and Figure 10. These give us information about the reliability with which we can determine the form of $F(x)$ by using the proposed new procedure. In particular, if we call q the pooled fraction of wrong determinations, Figure 9 shows a plot of $\log_{10} q$ as a function of $\log_{10} (NJ)$ for $NJ = 10, 20, 30$ and 40 . A straight-line fit to this data is also shown. The principal conclusion is that we can reduce q below .1 by taking NJ greater than about 40. However, this conclusion is only tentative until many more cases can be run.

The 12D estimates in cases W20, L20, W40 and L40 are shown in Table 4. The main conclusion is that the method produces good estimates of 12D provided the distribution is correctly identified, especially in the cases where $NJ = 40$. For example, the 18 trials of case W40 were all correctly identified as Weibull Distributions. The average estimate of 12D was 5.44 (the exact value being 5.38) and the standard deviation was .12. Of course, when the estimates are wrong, the results are poor. For instance, the four cases of L40 which were wrongly identified as Weibull Distributions had an average estimated 12D of 5.01 compared with the exact 12D of 5.96. This is rather bad, but even so it is slightly better than the average 12D estimated by the Schmidt-Nank Method from the tests at spore load 10^7 , which is about 4.8.

The comparison of the cases W40 and L40 with W120 and L120 is very striking and is shown in Table 5. The results provide strong evidence that, for the purpose of determining the distribution, it is better to test at several different spore loads than to test the same total number of cans all at one spore load. The fraction of wrong determinations is greater for the tests at one spore load with about 99% confidence.

TABLE 3

Summary of identifications of spore-distribution
function in various cases.

Case	No. of trials	No. of wrong determinations	Fraction of wrong determinations
W10	43	8	.186
L10	<u>43</u>	<u>15</u>	<u>.349</u>
Total, 10	86	23	.267
W20	36	4	.111
L20	<u>36</u>	<u>8</u>	<u>.222</u>
Total, 20	72	12	.167
W30	22	0	0
L30	<u>21</u>	<u>4</u>	<u>.190</u>
Total, 30	43	4	.093
W40	28	0	0
L40	<u>28</u>	<u>6</u>	<u>.214</u>
Total, 40	56	6	.107

TABLE 4

Statistics on the estimates of 12D in various cases.

Case	Correctly identified	No. of trials	Ave. of $x_c=12D$	Std. Dev. of $x_c=12D$
W20	Yes	32	5.49	.21
	No	<u>3</u>	<u>6.69</u>	<u>.34</u>
	Total	35	5.59	.40
L20	Yes	28	6.19	.24
	No	<u>8</u>	<u>5.17</u>	<u>.17</u>
	Total	36	5.96	.49
W40	Yes	18	5.44	.12
	No	<u>0</u>	<u>0</u>	<u>0</u>
	Total	18	5.44	.12
L40	Yes	14	6.05	.13
	No	<u>4</u>	<u>5.01</u>	<u>.16</u>
	Total	18	5.82	.46

TABLE 5

Statistics on wrong identification of distribution functions
in cases W40, L40, W120 and L120.

Case	No. of trials	Fraction of wrong identifications
W40	18	0/18
L40	18	4/18
Total	36	4/36
W120	32	16/32
L120	32	17/32
Total	64	33/64

Clearly we obtain much more reliable determinations of the distribution function by testing at several different spore loads than at one spore load. But, the results show even more than this. In the present example we are trying to decide whether an unknown distribution is of Weibull or lognormal form, given that it is one of the two. A pure guess, without any tests, would identify the distribution correctly for .50 of the trials. We see, therefore, that the tests at one spore load, which give the correct identification with a probability that 95% of the time lies between .38 and .64, are insignificantly more reliable than just guessing. So far as determining the distribution is concerned, it is an almost complete waste of time and money to run tests at one spore load.

Eight trials of the case E40 were run. Here the distributions $F(x)$ is of Weibull type, with $\beta = 1$ and $\eta = .165$, i.e. it is an exponential distribution whose exact 12D value is 4.56. The new method of analysis identified the distribution as of Weibull type in all eight cases and gave the following as the average and standard deviation of the estimated quantities

	ave.	st'd. dev.	exact value
β	.961	.037	1.000
η	.150	.016	.165
12D = x_c	4.73	.150	4.56

Figure 10 shows the averages and standard deviations of the 12D values estimated by the Schmidt-Nank method for each dose from the data for $n = 10^7$.

Both methods give estimates of 12D that are sufficiently accurate for practical purposes. Figure 10 shows that the Schmidt-Nank 12D estimates from the lower end of the partial spoilage dose range will tend to be too low. The estimates for $x = 3.4$ and 3.6 are not very meaningful because they are based on few partial spoilage points, and the effect of noise on those points is quite severe.

VIII. Conclusions and Remarks

In this Section we summarize the main conclusions of this paper.

- (i) The present method of conducting inoculated pack experiments and calculating the results can be improved by taking the following steps:
 - (ia) The inoculated pack design should include tests at several different spore loads, and
 - (ib) The Schmidt-Nank procedure for analyzing the data should be replaced by the method described in Section (V).
- (ii) If this is done, the results of Section (VI) show tentatively that tests of a reasonable number of cans (720) have a rather good probability (.9 or better) of correctly identifying the distribution functions and accurately estimating the 12D dose.
- (iii) The available experimental results suggest that the distribution of spore resistance is of either Weibull or lognormal type. That does not preclude its being of exponential form, which is a special case of the Weibull distribution.
- (iv) In designing the tests it is advantageous to use dose increments that are small enough so that four or more partial spoilage data points are obtained at each spore load. Increments of .2 megarads, which were used in the Monte Carlo simulation, will often accomplish this. The errors in the doses may occasionally cause overlapping of the ostensible dose regions, but the evidence of the simulation is that this is not a serious disadvantage.
- (v) A much more extensive series of Monte Carlo simulations should be run, to obtain reliable quantitative estimates of the numbers of cans and doses. Then the suggested method should be tried out in an actual experiment to see whether the benefits are diluted by any practical difficulties. If the experiment is to be a real test of the accuracy of the method, it must be carried out under conditions where the true distribution function and 12D dose of the organism are known. Otherwise there is no way to check the results of the new method.

- (vi) Even under the best of conditions, there is still a (small) probability that an inoculated pack will lead to a wrong determination of distribution function and a very inaccurate 12D. This is unavoidable.
- (vii) It is plain that the difficulty in this problem stems ultimately from the vast extrapolation that has to be done in estimating the MRD. Some thought should be given to other experimental approaches, or, ultimately, to other safety criteria.

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Appendix A: Proof that D increases when $\alpha_n U_n \leq \log_e n$.

In this Appendix we shall derive the condition that must be satisfied in order (see Equation (23)), that

$$A(x) = \log_e n - \log_e [1 - \Phi(x)] - x [1 - \Phi(x)]^{-1} (d\Phi/dx) \quad (A.1)$$

be an increasing function of x throughout the partial spoilage range. In the partial spoilage range we shall use Formulas (10) and (11), in the forms

$$x = U_n + \alpha_n^{-1} y \quad (A.2)$$

$$\Phi = \exp(-e^{-y}) \quad (A.3)$$

$$d\Phi/dx = \alpha_n e^{-y} \Phi. \quad (A.4)$$

If we combine these with (A.1) we obtain

$$A = \log_e n + T - W \alpha_n U_n \quad (A.5)$$

where

$$T = -\log_e (1 - \Phi) + (-y) \Phi e^{-y} (1 - \Phi)^{-1} \quad (A.6)$$

$$W = \Phi e^{-y} (1 - \Phi)^{-1} \quad (A.7)$$

We shall study the behavior of T and W separately, using the following easily verified relations,

$$\Phi e^{-y} \rightarrow 0 \quad \text{as } y \rightarrow -\infty \quad (A.8)$$

$$(-y) \Phi e^{-y} \rightarrow 0 \quad \text{as } y \rightarrow -\infty \quad (A.9)$$

$$(1 - \Phi)^{-1} e^{-y} > 1 \quad \text{for all } y. \quad (A.10)$$

First we investigate W . By direct calculation

$$dW/dy = \Phi(1-\Phi)^{-1} \left\{ -1 + e^{-Y} (1-\Phi)^{-1} \right\} e^{-Y} > 0, \quad (A.11)$$

and we see that W increases with y for all y . As $y \rightarrow -\infty$, we have $1-\Phi \rightarrow 1$, and therefore Equations (A.7) and (A.8) show that

$$W \rightarrow 0 \quad \text{as} \quad y \rightarrow -\infty.$$

As $y \rightarrow +\infty$, we set $\epsilon = e^{-Y} \ll 1$ and obtain

$$\begin{aligned} \Phi &= e^{-\epsilon} \approx 1 - \epsilon + \frac{\epsilon^2}{2} = 1 - \epsilon \left(1 - \frac{\epsilon}{2}\right) \\ W &\approx \epsilon(1-\epsilon)/\epsilon = 1 - e^{-Y} \end{aligned}$$

Hence

$$W \rightarrow 1 \quad \text{as} \quad y \rightarrow \infty,$$

and W increases monotonically from zero to unity as y increases from $-\infty$ to $+\infty$.

The behavior of T is only slightly more complicated. We may differentiate (A.6) and find that

$$dT/dy = -y dW/dy, \quad (A.12)$$

whence it is plain that T attains a maximum at $y = 0$ and has no other extrema. Equations (A.9) and (A.6) show that

$$T \rightarrow 0 \quad \text{as} \quad y \rightarrow -\infty.$$

As $y \rightarrow +\infty$, we set $\epsilon = e^{-Y} \ll 1$ again and

$$\begin{aligned} T &\approx -\ln \left[\epsilon \left(1 - \frac{\epsilon}{2}\right) \right] + \frac{(\ln \epsilon) (1 - \epsilon) \epsilon}{\epsilon} \\ T &\approx -\ln \left(1 - \frac{\epsilon}{2}\right) - \epsilon \ln \epsilon \approx \frac{\epsilon}{2} - \epsilon \ln \epsilon \end{aligned}$$

Therefore

$$T \rightarrow 0 \quad \text{as} \quad y \rightarrow +\infty.$$

T increases from zero to its maximum value,

$$T(0) = -\ln [1 - \Phi(0)] = -\ln (1 - e^{-1}) = .459,$$

as y increases from $-\infty$ to zero, then T decreases back to zero as y increases from zero to $+\infty$.

Differentiating (A.5), and using (A.11), (A.12) and (A.2), we get

$$dA/dy = - (y + \alpha_n U_n) dW/dy = - \alpha_n x dW/dy < 0.$$

Also, since both T and W tend to zero as $y \rightarrow -\infty$,

$$A \rightarrow \log_e n \quad \text{as} \quad y \rightarrow -\infty, \text{ i.e. } x \ll U_n,$$

and, since $W \rightarrow 1$ and $T \rightarrow 0$ as $y \rightarrow +\infty$,

$$A \rightarrow \log_e n - \alpha_n U_n \quad \text{as} \quad y \rightarrow +\infty, \text{ i.e. } x \gg U_n.$$

Hence we see that A decreases monotonically from $\log_e n$ to $\log_e n - \alpha_n U_n$ as x increases through the partial spoilage range. Hence $A > 0$ everywhere in the partial spoilage range, and so D increases through the partial spoilage range, if and only if

$$\alpha_n U_n \leq \log_e n.$$

If

$$\alpha_n U_n > \log_e n,$$

the foregoing discussion shows that D increases at the lower end of the partial spoilage range, reaches a maximum somewhere and decreases in the upper part of the range.

Appendix B: The Least-Squares Polynomial Estimation.

In this Appendix we present the formulas appropriate for the Least-Square fit of a second-degree polynomial to the transformed data, which consists of points (y_j, t_j) $j = 1, 2, \dots, M$. In this presentation we use the familiar ideas of orthogonal polynomials.

The inner product of two functions, f and g , taking the values f_j and g_j respectively at points $t = t_j$, is defined by

$$\langle f, g \rangle = \sum_{j=1}^M f_j g_j.$$

f and g are said to be orthogonal if

$$\langle f, g \rangle = 0.$$

Further, in terms of the shifted independent variable

$$\begin{aligned} x_j &= t_j - \bar{t} \\ \bar{t} &= M^{-1} \sum_{j=1}^M t_j \end{aligned}$$

we define the following constants

$$\beta_k = \sum_{j=1}^M x_j^k \quad K = 1, 2, 3, 4$$

$$\lambda = \beta_4 - \beta_3^2 \beta_2^{-1} - \beta_2^2 \beta_0^{-1}$$

Notice that

$$\beta_0 = M, \quad \beta_1 = 0.$$

It may be verified that the three polynomials, ϕ_0 , ϕ_1 and ϕ_2 , defined below,

$$\phi_0(x) = \beta_0^{-1/2}$$

$$\phi_1(x) = \beta_2^{-1/2} x$$

$$\phi_2(x) = \lambda^{-1/2} \{ x^2 - (\beta_3/\beta_2) x - (\beta_2/\beta_0) \}$$

satisfy the orthogonality conditions

$$\langle \phi_i, \phi_k \rangle = 0 \quad i \neq k \quad ; \quad i = 0, 1, 2; \quad k = 0, 1, 2$$

and the normalization conditions

$$\langle \phi_k, \phi_k \rangle = 1 \quad k = 0, 1, 2.$$

The second-degree polynomial approximation to the data is given by

$$y = c_0 \phi_0(x) + c_1 \phi_1(x) + c_2 \phi_2(x) \quad (B.1)$$

and the values of c_0 , c_1 and c_2 estimated by the Least-Squares procedure are

$$\hat{c}_0 = \beta_0^{-1/2} \sum_1^M y_j$$

$$\hat{c}_1 = \beta_2^{-1/2} \sum_1^M x_j y_j$$

$$\hat{c}_2 = \lambda^{-1/2} \{ \sum y_j x_j^2 - (\beta_3/\beta_2) \sum y_j x_j - (\beta_2/\beta_0) \sum y_j \}$$

Thus the best second degree polynomial approximation in the least-squares sense to the given data is

$$y = \hat{c}_0 \beta_0^{-1/2} + \hat{c}_1 \beta_2^{-1/2} x + \hat{c}_2 \lambda^{-1/2} \{ x^2 - (\beta_3/\beta_2) x - (\beta_2/\beta_0) \}$$

The curvature measure, K , used in (31), (32) and (35), is calculated from

$$K = \frac{1}{2} \frac{d^2 y}{dt^2} = \frac{1}{2} \frac{d^2 y}{dx^2} = c_2 \lambda^{-1/2}. \quad (B.2)$$

We see that the curvature is simply proportional to \hat{c}_2 . If the data lies on a straight line,

$$\hat{c}_2 = 0 \quad \text{and} \quad K = 0.$$

In that case

$$y = c_0 \beta_0^{-1/2} + c_1 \beta_2^{-1/2} (t - \bar{t})$$

and the constants A and B that occur in Equation (31) to (34) are given by

$$A = c_0 \beta_0^{-1/2} - c_1 \beta_2^{-1/2} \bar{t} \quad (B.3)$$

$$B = c_1 \beta_2^{-1/2} \quad (B.4)$$

To study the errors in this analyses, we define, ϵ_j as the error in the approximate formula (B.1) at the point $t = t_j$. We assume, as usual, that the ϵ_j are independent, normal random variables with zero mean and variance σ^2 . Then the best estimate of σ^2 is given by

$$S^2 = (M-3)^{-1} \sum_{j=1}^M \{\epsilon_j\}^2$$

and this S is the one used in Equations (35). It is known that under these assumptions the quantity

$$(c_i - \hat{c}_i) / S \quad i = 0, 1, 2$$

obeys a Student t - distribution with M-3 degrees of freedom. This permits us to make confidence interval statements about C_i if we wish to. In particular the central confidence interval for $K = c_2 \lambda^{-1/2}$, for a confidence coefficient $(1 - \alpha)$, is

$$\lambda^{-1/2} \left\{ \hat{c}_2 \pm S t_{1-(\alpha/2)} \right\}$$

With the aid of this formula we can decide in any practical case whether the difference between K_w and K_L is meaningful or not. The quantity ρ , defined in Equation (35), is a related measure of the significant curvature.

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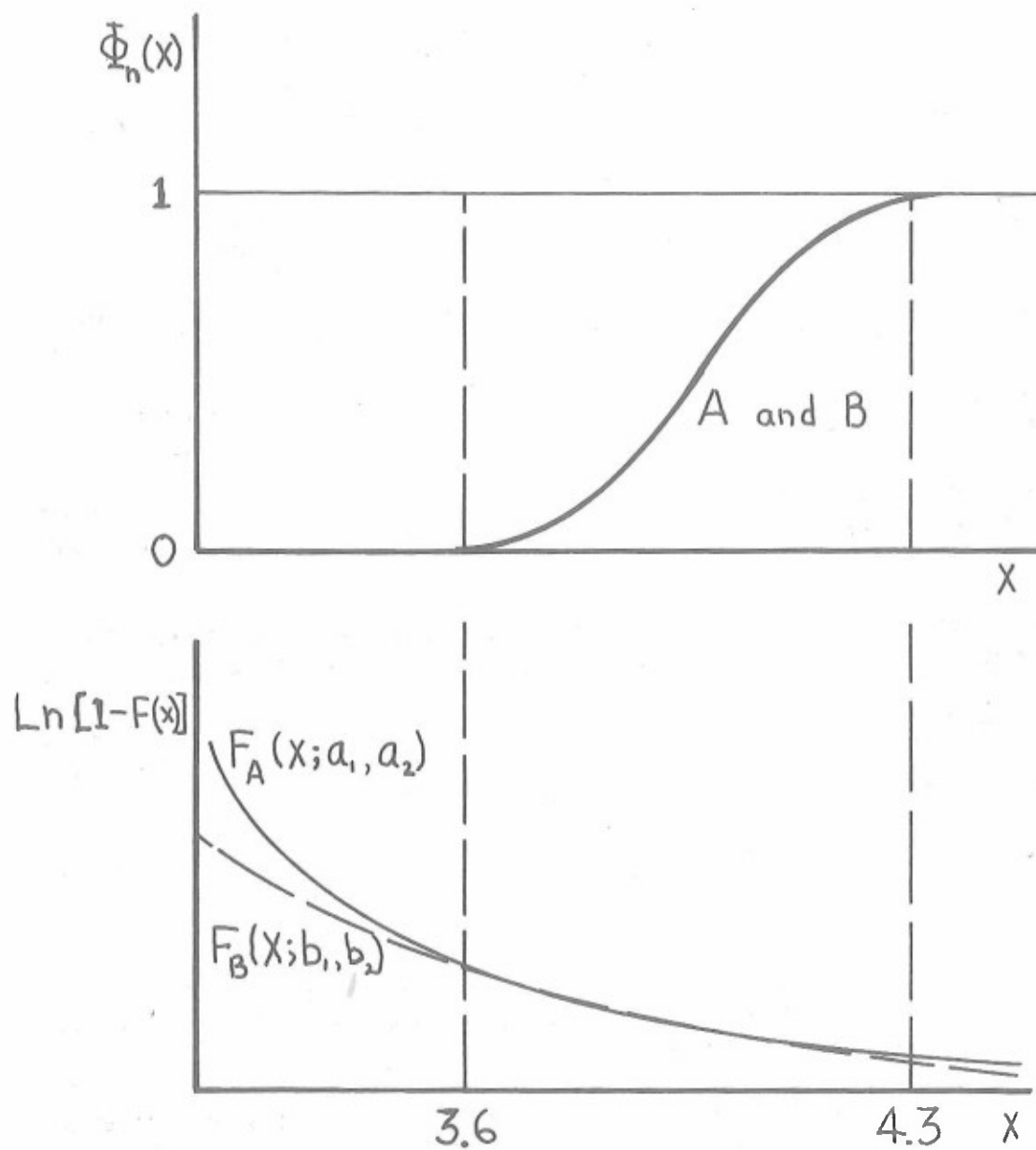


Fig. 1: Sketch of two distributions that nearly coincide in the region $3.6 \leq x \leq 4.3$.

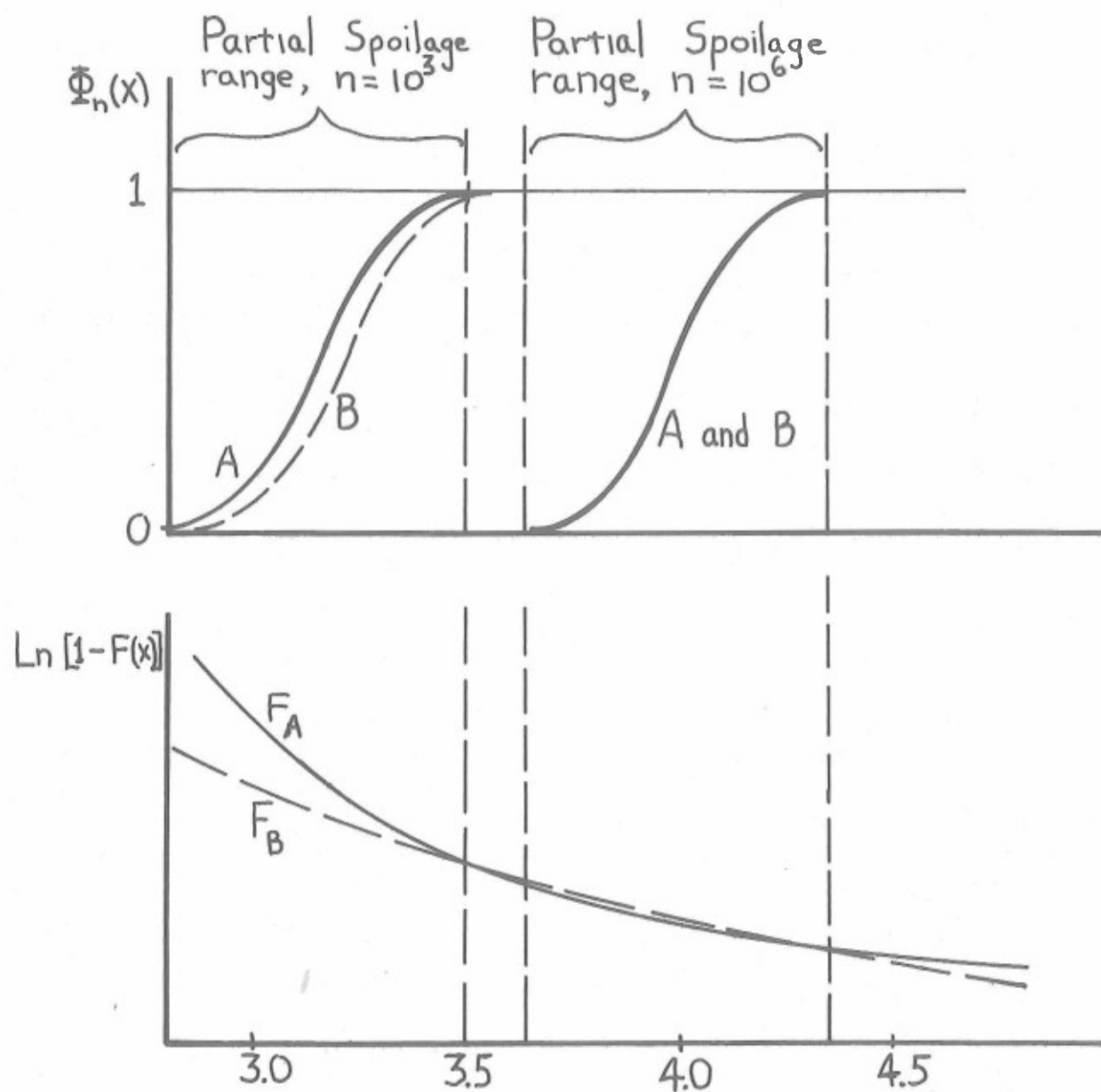


Fig. 2: Schematic showing partial spoilage ranges for $n = 10^3$ and $n = 10^6$.

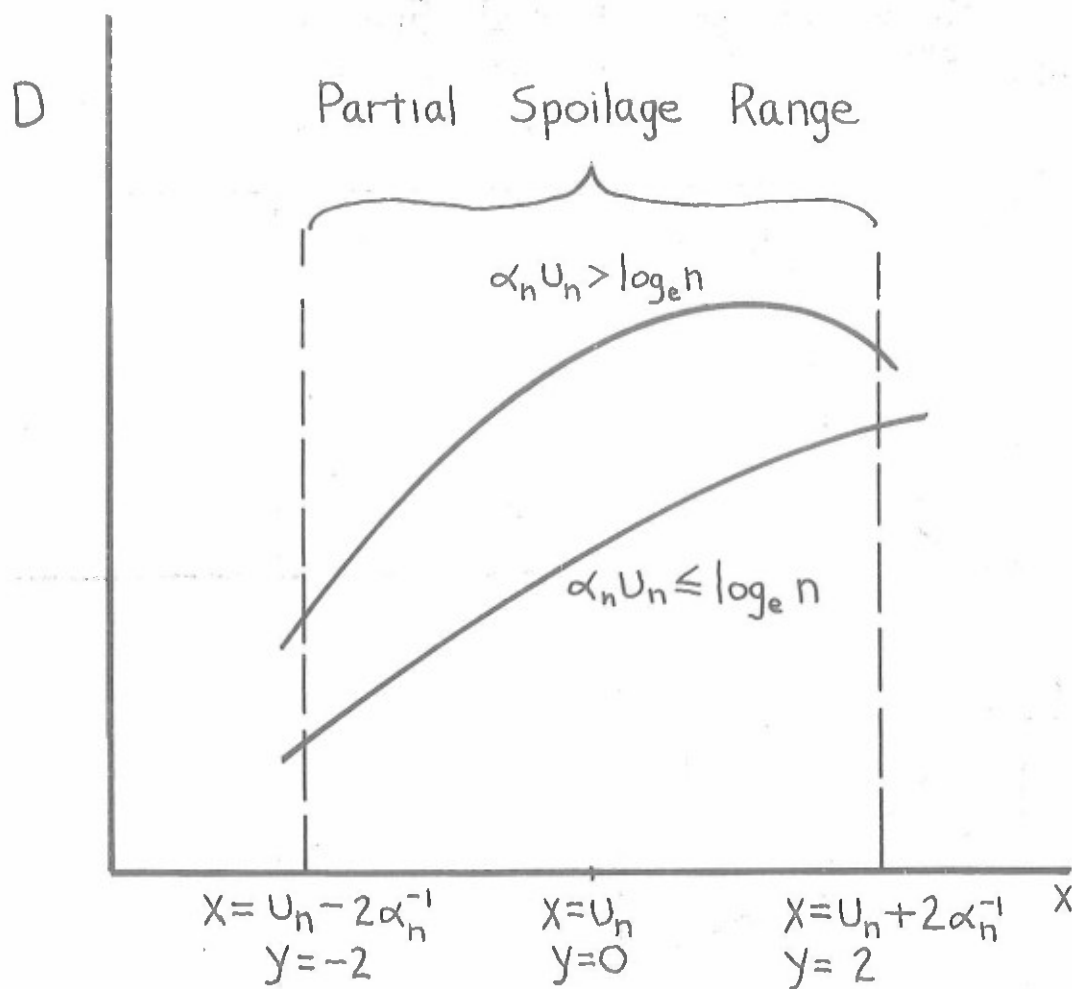


Fig. 3: Schematic behavior of $D(x)$ in partial spoilage range.

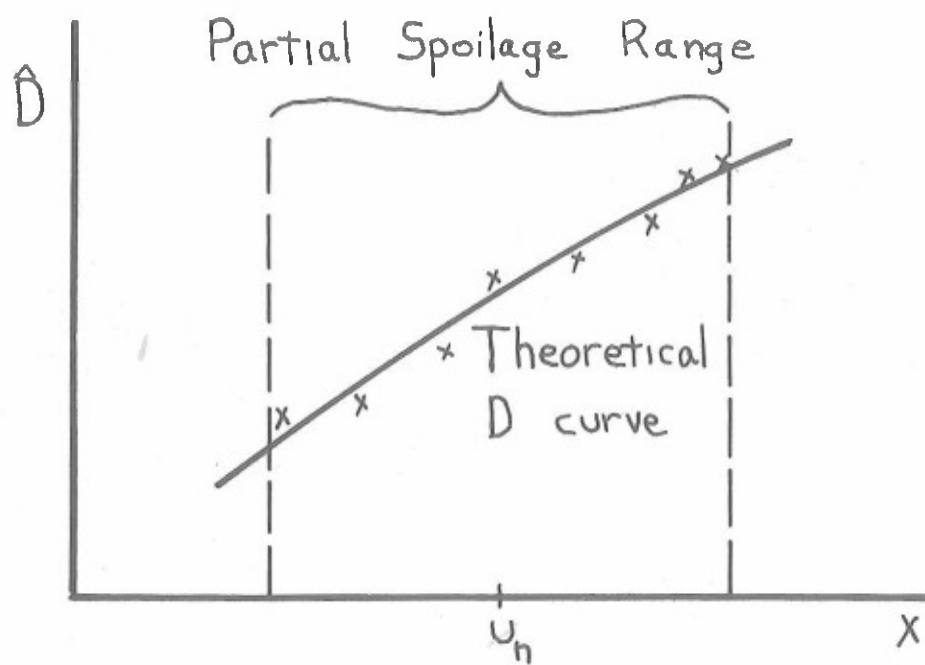
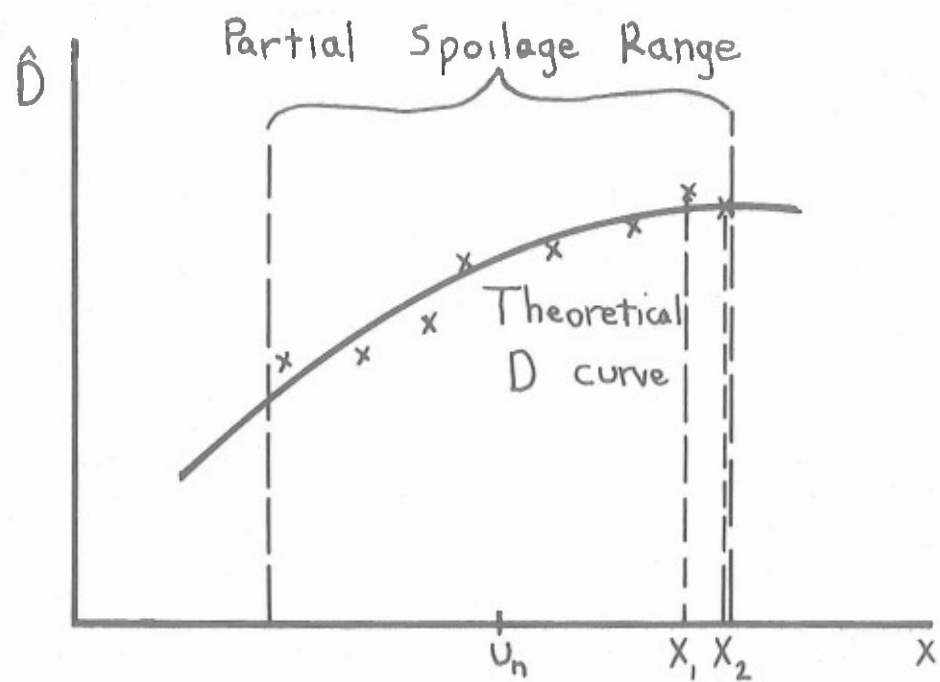


Fig. 4: Effect of random noise on plots of D vs x . The points x mark experimental values of D .

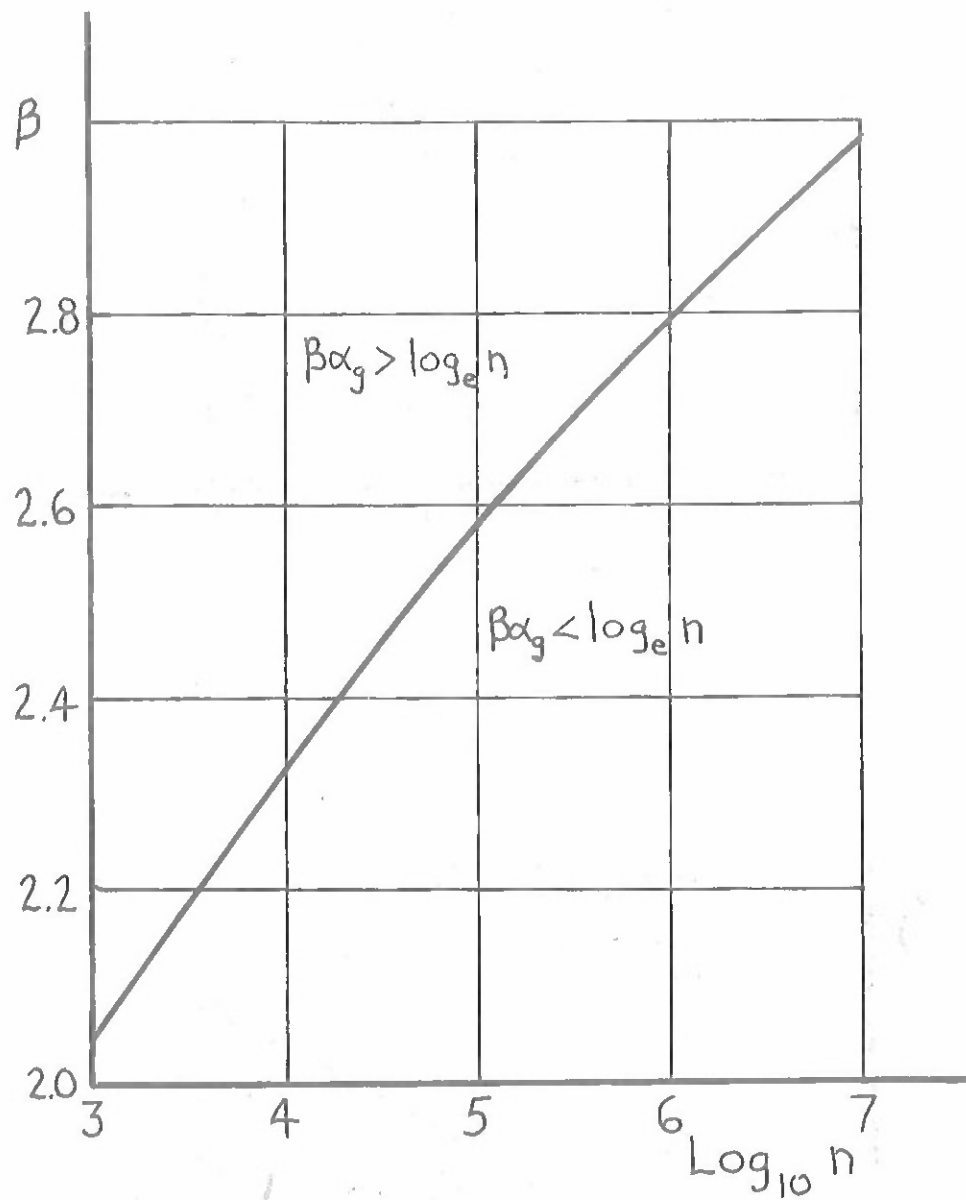


Fig. 5: Acceptable range of lognormal constant, β , as a function of n .

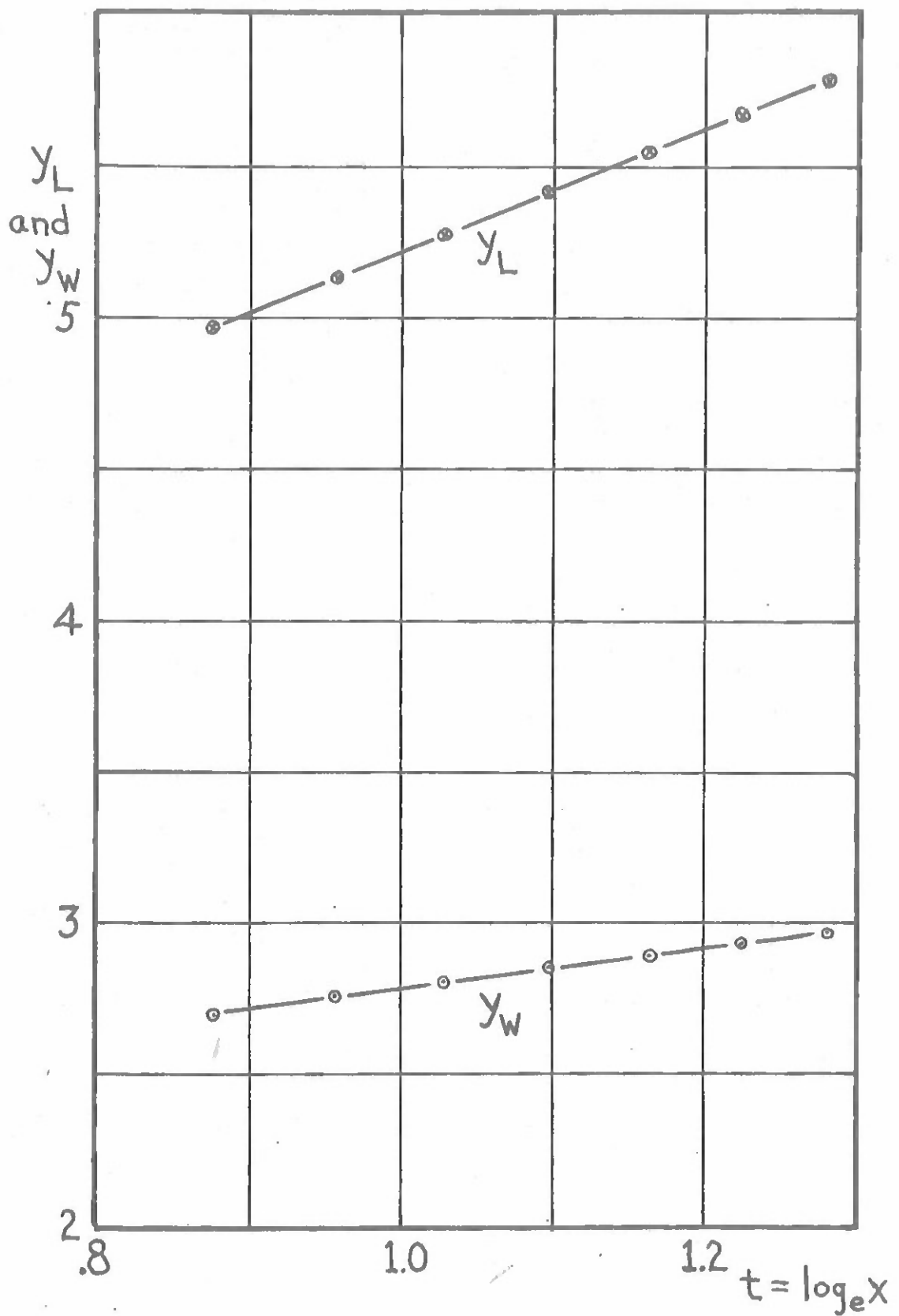


Fig. 6: Graphs of lognormal and Weibull plots of data derived from a lognormal distribution for $n = 10^7$.

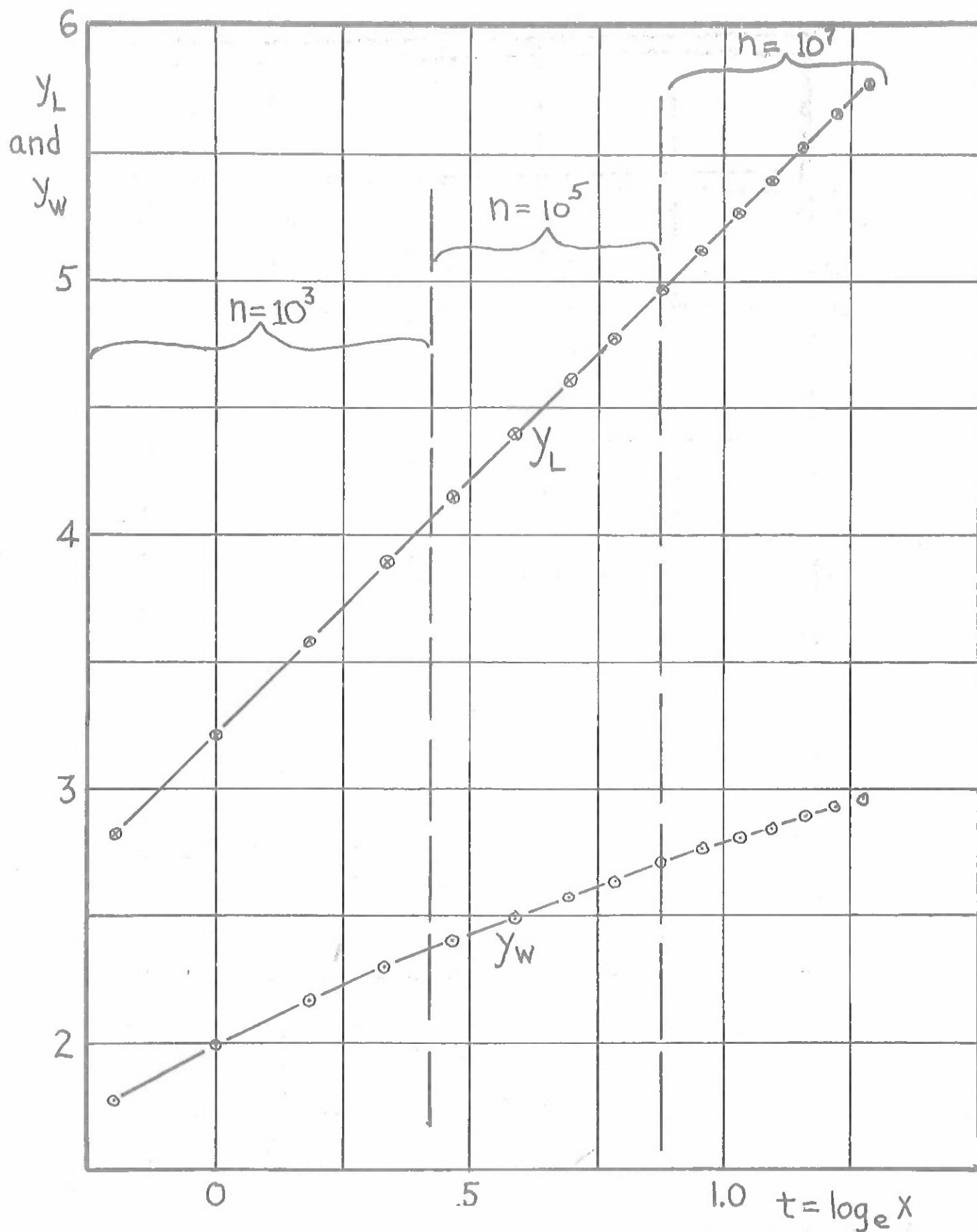


Fig. 7: Graphs of lognormal and Weibull plots of data derived from a lognormal distribution for $n = 10^3$, 10^5 and 10^7 .

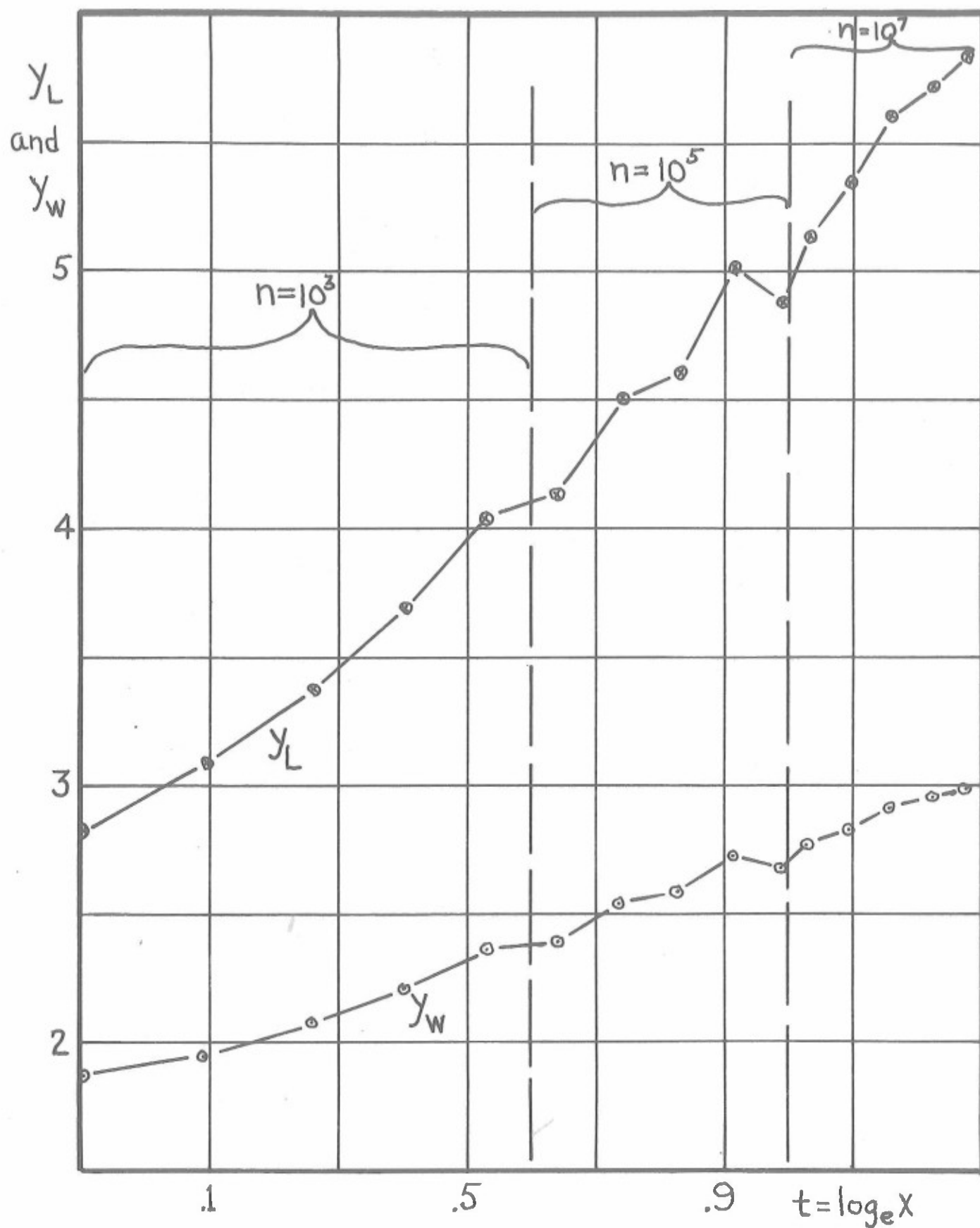


Fig. 8: Weibull and lognormal plots of typical artificial data generated by the simulation procedure.

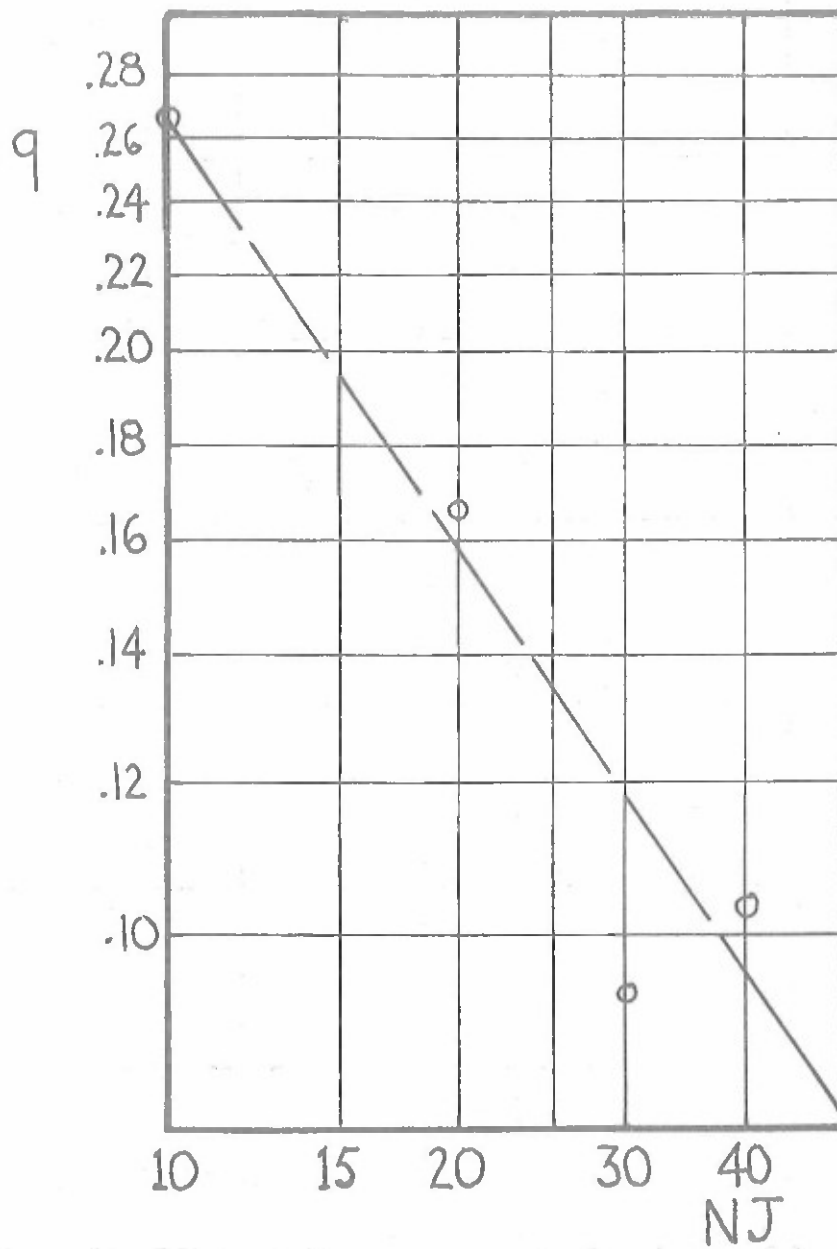


Fig. 9: Logarithmic plot of the experimental probability of correctly determining $F(x)$.

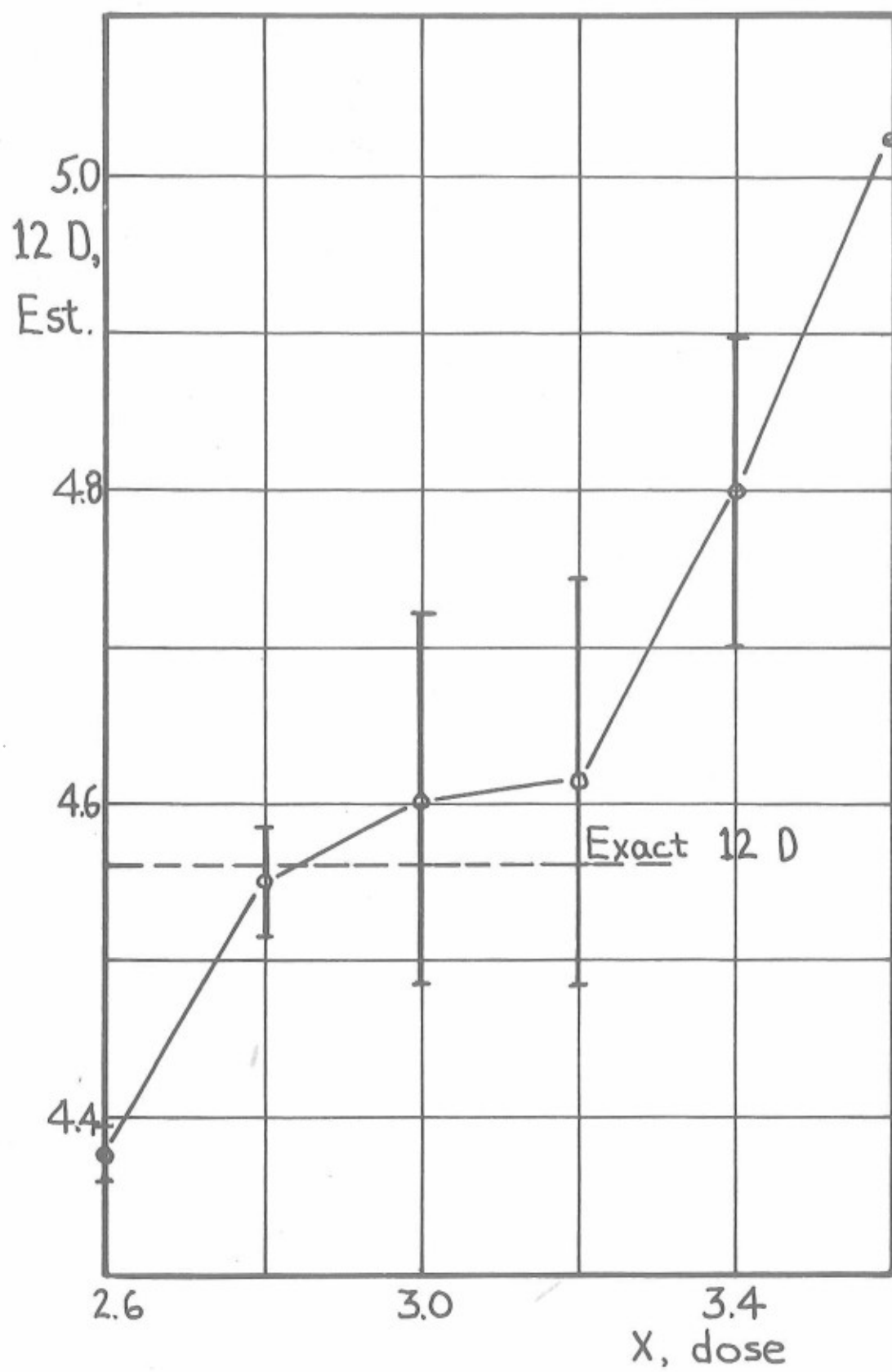


Fig. 10: The 12D estimated by the Schmidt-Nank formula for an Exponential $F(x)$.

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This report presents a mathematical analysis of the methods used for determining the effectiveness of radappertization (radiation-sterilization) of food. A general theory is developed which makes it clear that two inter-related distribution functions, the probability of organism death and the probability of can-sterilization, play important parts in the process. A critique is given of the Schmidt-Nank method for calculating the 12D dose and the implications of the experimental data are studied. Modifications in both experimental design and data analysis are proposed. These are evaluated by using them to analyze artificial data generated by a fairly realistic computer-simulation model. The proposed methods give considerably more accurate results than the traditional one, and it is concluded that the new methods appear promising for future use.

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